METHOD FOR OBTAINING A CELLULAR PRODUCT FROM A CELL OR A TISSUE USING PROTEIN-FREE MEDIUM

CROSS REFERENCE TO RELATED APPLICATIONS

This application is divisional of U.S. patent application Ser. No. 11/150,322, filed 13 Jun. 2005, used as U.S. Pat. No. 7,611,896, which is a continuation-in-part of U.S. patent application Ser. No. 10/990,971, filed 18 Nov. 2004, issued as U.S. Pat. No. 7,452,721, which is a continuation of U.S. patent application Ser. No. 10/173,586, filed 18 Jun. 2002, issued as U.S. Pat. No. 7,008,774, which is a continuation of U.S. patent application Ser. No. 09/725,182, filed 29 Nov. 2000, now abandoned, which claims the benefit of U.S. Provisional Patent Application No. 60/168,300, filed 1 Dec. 1999, all of which are herein incorporated by reference.

ACKNOWLEDGMENT OF GOVERNMENT SUPPORT

This invention was made by employees of the United States Army. The government has rights in the invention.

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates generally to a method of diagnosing leishmaniasis in a subject suspected of being infected with the parasitic protozoa *Leishmania*. In particular, the invention relates to enzyme-linked immunosorbent assays (ELISAs) for the detection of *Leishmania* parasite circulating antigens and *Leishmania*-specific antibodies in host samples.

2. Description of the Related Art

Leishmaniasis is a serious and sometimes fatal disease estimated by the World Health Organization (WHO) to affect approximately 12 million people in 88 countries. Recent epidemics of leishmaniasis have occurred in the Africa, the Indian subcontinent and Brazil. The significant morbidity and mortality caused by leishmaniasis is a cause for concern in endemic areas. Such concern has more recently expanded to include non-endemic areas because of the increase in global travel concomitant with the increased incidence of the disease in HIV-infected and intravenous drug-user populations.

Unfortunately, current acceptable diagnostic practices lack the means for efficiently and accurately identifying those infected or exposed to the disease-causing parasite as explained in Martin, S. et al. (1998) Military Medicine 163 (23):801-807. As a result, the prevention of leishmanial epidemics is greatly hindered and patient management is difficult. Additionally, there is an imminent threat of HIV and *Leishmania* co-infection, a more malignant infection that is difficult to diagnose and treat. To date, there are no antigen-detection type diagnostic tests available for leishmaniasis. In view of this, antigen-detection assays are desperately needed for diagnosis, patient management and epidemiological studies.

Recombinant kinesin protein, rK39, is one of the few antigens that have been used in the development of antibody-detection immunoassays for active visceral leishmaniasis (VL). However, assays developed with this antigen and others fail to consistently detect antibodies in other clinical syndromes associated with a predominately T-cell and muted B-cell response. Moreover, antibody-detection assays have an inherent dependence on the immune response of the host to the parasite antigen which significantly diminishes its use.

For example, in HIV/AIDS and other immuno-compromised conditions, the infection may not produce proportional antibody production and thereby escape detection.

Generally, the serological tests for diagnosing active VL are highly sensitive (>90%). See Senaldi, G., et al., (1996) J. Immunol. Methods 193:9-5. These serological tests, however, pose problems of non-specificity resulting in false positive results from reference samples of other infectious diseases. Modifications of the antigens used for direct agglutination test (DAT) and for ELISA in eliminating false-positive results have met variably success. See Zijlstra, E. E., et al., (1997) Trans. R. Soc. Trop. Med. Hyg. 91:671-673 and Badaro, R., et al., (1996) J. Inf. Dis. 173:758-761. Unfortunately, serological tests are rarely performed to diagnose cutaneous leishmaniasis, the most common form of the disease, because the sensitivities and specificities are disappointingly low for this clinical manifestation. See Sanchez, J. L., et al., (1992) Am. J. Trop. Med. Hyg. 47:47-54; Garcia-Miss, M. R., et al., (1990) Trans. R. Soc. Trop. Med. Hg. 84:356-358.

The antigens used in immunoassays for the detection of leishmaniasis are traditionally derived from promastigotes cultivated in vitro, or from recombinant proteins. See Badaro et al. (1996); Choudhary, S., et al., (1992) J. Comm. Dis. 24:32-36; Badaro, R., et al., (1986) Am. J. Trop. Med. Hyg. 35:72-78; Choudhary, A., et al., (1990) Trans. R. Soc. Trop. Med. Hyg. 84:363-366; and Reed, S. G., et al., (1990) Am. J. Trop. Med. Hyg. 43:632-639. However, promastigotes shed, excrete and secrete products into the culture medium to produce conditioned medium. These released products or exo-antigens are immunogenic to the host. See Schnur, L. F., et al., (1972) Isrl. J. Med. Sci. 8:932-942; Sergeiev, V. P., et al., (1969) Med. Parasitol. 38:208-212; El-On, J., et al., (1979) Exper. Parasitol. 47:254-269; and Bray, R. S., et al., (1966) Trans. R. Soc. Trop. Med. Hyg. 60:605-609.

In the prior art assays these exo-antigens are released into in vitro culture medium containing serum. Thus, the presence of complex proteins or serum components required for growth of the parasites in the culture medium pose several problems in the prior art assays. For example, the amount of manipulation needed to purify the targeted antigens from spent media can affect the native composition of certain components necessary for a highly sensitive assay. Furthermore, insufficient purification of parasite products may create problems with specificity as serum proteins contaminants in the antigen preparations, cause non-specific downstream reactions. Generally, the prior art assays are limited in scope to one species complex or clinical manifestation and have never demonstrated combined sensitivity and specificity of more than 90%.

Thus, a need exists for highly sensitive and highly specific assays for screening for exposure or diagnosing *Leishmania* infections.

SUMMARY OF THE INVENTION

The invention relates to an immunoassay for detecting IgM and IgG antibodies in a sample from a subject having visceral, cutaneous or canine leishmaniasis.

The invention also relates to an immunoassay for detecting *Leishmania* parasite circulating antigens in a sample from a subject having visceral, cutaneous or canine leishmaniasis.

In one embodiment, the invention relates to a leishmaniasis immunoassay, which is based on soluble antigens from promastigotes cultivated in a protein-free and serum-free medium.